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Occurrence and risk assessment of phenol and substituted phenols in water and fish collected from the streams in eastern Gangwon State, Korea

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Abstract: An analytical method was developed for the determination of phenol (P) and the seven substituted phenols in water samples and fish tissue samples collected from three streams located in eastern Gangwon State in spring and summer. The phenols were extracted and then derivatized to phenyl acetates using acetic anhydride. The derivatives were subsequently identified and quantified using gas chromatography coupled with mass spectrometry. P and 4-nitrophenol (4NP) were found at relatively high levels in water, ranging from below the method detection limit (MDL) to 3.32 μg/L and from < MDL to 4.91 μg/L, respectively. P and 4NP were also the dominant compounds in the fish tissue, ranging from < MDL to 407 μg/kg and from < MDL to 870 μg/kg, respectively. Phenol concentrations were significantly higher in spring than in summer. The ecological risk quotient calculated for P was higher than 4NP but not high enough to pose any risk of adverse effects to fish health.

Key words: phenols, GC-MS, derivatization, water, fish tissue

1. Introduction

Phenols are volatile organic compounds that have a hydroxyl group attached to an aromatic hydrocarbon. These compounds present in the environment generally derive from agricultural farmland, industrial activity, domestic waste, pulp mills, wood treatment facilities, sewage treatment plants, and leachate from waste sites and landfills.[1] They are also generated by the decomposition of natural organic matter such as humic substances, tannins, lignins, dead animals, and microorganisms.[2] The phenols produced by these sources can subsequently be transported into water bodies by rain.[3]

Phenols can be present in the environment as a result of contamination. They can react with chlorine or nitrate ions to form chlorophenols or nitrophenols. The major sources of chlorophenols in surface water are industrial waste and leaching from the soil. Some chlorophenols are intermediate metabolites

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of microbial degradation of substances such as chlorophenoxyacetic acid herbicides,[4] while others originate from chlorine-based disinfection in water treatment systems[5] and via the chlorination of mono- and polyaromatic compounds in the environ- ment.[6] Mono-substituted nitrophenols such as 2-nitrophenol (2NP) and 4-nitrophenol (4NP) are formed via the nitration of phenol with a nitrate ion under ultraviolet irradiation over a wide pH range. They are used as materials or precursors for industrial processes such as polymers and pharmaceuticals and can be artificially introduced into the environment. Nitrophenols in the air, produced by the reaction of hydroxyl radicals with nitrate ions, can migrate into water by rainfall. However, because nitrophenols are metabolized as glucuronide and sulfate conjugates and excreted via the urine within 48 h, their bioaccumulation in living organisms is rare.[6,7]

The solubility of phenols in water is dependent on the degree of substitution of the benzene rings. Phenols substituted with electron-withdrawing groups such as nitro and chloro-groups are less hydrophilic than phenol (P), whereas those substituted with electron-donating groups such as methyl groups are more hydrophilic. Therefore, P, 2,4-dimethylphenol (24DMP), and 4-chloro-3-methylphenol (4C3MP) are more likely to be detected in water, whereas 2,4-dichlorophenol (24DCP), 2NP, 2,4,6-trichlorophenol (246TCP), 4NP, and pentachlorophenol (PCP), which are more hydrophobic than P, are more readily partitioned into organic matter-rich soil and sediment.[2]

Human consumption of phenols may have harmful health effects on the bone marrow, blood, liver, kidneys, muscles, and eyes and cause cardiac depression, necrosis, and skin irritation.[3] Other toxicological endpoints associated with chlorophenols include T-lymphocyte dysfunction, carcinoma, and sarcoma.[8] In addition, phenols can cause harmful effects on aquatic organisms and higher trophic levels due to their accumulation potential.[9] Given the adverse effects of phenols, the US Environmental Protection Agency (EPA) has listed P and other phenols (24DMP, 4C3MP, 24DCP, 2NP, 246TCP, 4NP, and PCP) as part of its list of 126 priority pollutants.[10] Phenols also have serious adverse effects on aquatic organisms, including genotoxicity, growth inhibition, and carcinogenicity.[11-13] For these reasons, ecological risk assessments for phenols are vital. An ecological risk assessment evaluates the possibility of adverse ecological effects arising from one or more stressors, and are used to regulate hazardous substances and protect ecosystem.[14]

Phenols have been found in the Buffalo River on the Eastern Cape of South Africa, which receives various forms of hazardous waste from an old tannery textile mill and a waste dump site, with the harbor area of the Buffalo River estuary recording the highest concentrations.[3] The most abundant compounds at all locations were P, 2-chlorophenol (2CP), and 2NP, and the highest concentrations were recorded for 2NP (1.22 μg/L and 0.54 μg/L in summer and fall, respectively). Zhong *et al.* (2018) reported that P, 2NP, 4NP, and 4-chlorophenol were found in three rivers in Tianjin, China (average concentration of 0.08-3.58 μg/L), with the highest concentrations for P in the dry season.[15] However, not many studies on the occurrence of these phenols have been conducted elsewhere.

Analytical techniques employed for the determination of phenols in water are quite diverse, including not only gas chromatography (GC) coupled with electron capture detection (ECD), flame ionization detection (FID), and mass spectrometry (MS) as detectors, but also high-performance liquid chromatography in combination with ultraviolet radiation or electrochemical detection.[8] GC methods are generally preferred with or without derivatization steps. EPA method 528 uses GC-MS following solid phase extraction without derivatization,[16] while EPA method 604 employs liquid–liquid extraction using dichloromethane and GC-FID without derivatization or GC-ECD with derivatization using pentafluorobenzyl bromide.[17] EPA method 8041A describes the analysis of sewage water using GC-FID with anisole derivatives or without derivatization or using GC-ECD with pentafluorobenzyl derivatives.[18] However, not many studies have investigated the most useful methods for the measurement of phenols in biological samples such as fish tissue.[19]

This study aimed to develop an analytical method

for the simultaneous determination of eight phenols (P, 24DMP, 4C3MP, 24DCP, 2NP, 246TCP, 4NP, and PCP) in water and fish using GC-MS following derivatization to phenyl acetates using acetic anhydride as a derivatizing reagent. The methods were used to test water and fish samples collected from the cities in Gangwon State for phenols, where a phenol spill occurred in 2013. and an ecological risk assessment was conducted for exposure to phenols within the aquatic environment.

2. Experimental

2.1. Chemicals and reagents

Standard P, 24DMP, 4C3MP, 24DCP, 2NP, 246TCP, 4NP, and PCP reagents were purchased from Supelco (Bellefonte, PA, USA). *o*-Cresol (C), which was used as a surrogate standard (SS), potassium carbonate, and acetic anhydride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride and sulfuric acid were supplied from Junsei Chemical (Tokyo, Japan) and Showa Chemical (Tokyo, Japan), respectively. Methanol, dichloromethane (DCM), ethyl acetate, and methyl *tert*-butyl ether (MTBE) were purchased from Tedia (Fairfield, OH, USA). Sodium thiosulfate, sodium sulfate, and *n*-butyl acetate were obtained from Daejung Chemicals & Metals (Siheung, Korea). Individual stock solutions of all target compounds and the SS were prepared in methanol. A standard mixture solution (1 mg/L) was prepared by diluting the individual standard solutions in methanol. The SS solution was also prepared in methanol (1 mg/L).

2.2. Study sites and sampling

The study sites in Gangwon State, Korea, are presented in *Fig.* 1. Ten sampling sites were selected for the collection of water and fish samples: Y1-3, O1-4, and J1-3. Sites O1-4 were in Nakpung and Jusu Streams (Okgye-myeon, Gangneung), near which 27 tons of phenol and 267 tons of petroleum chemicals leaked from the water circulation system of a

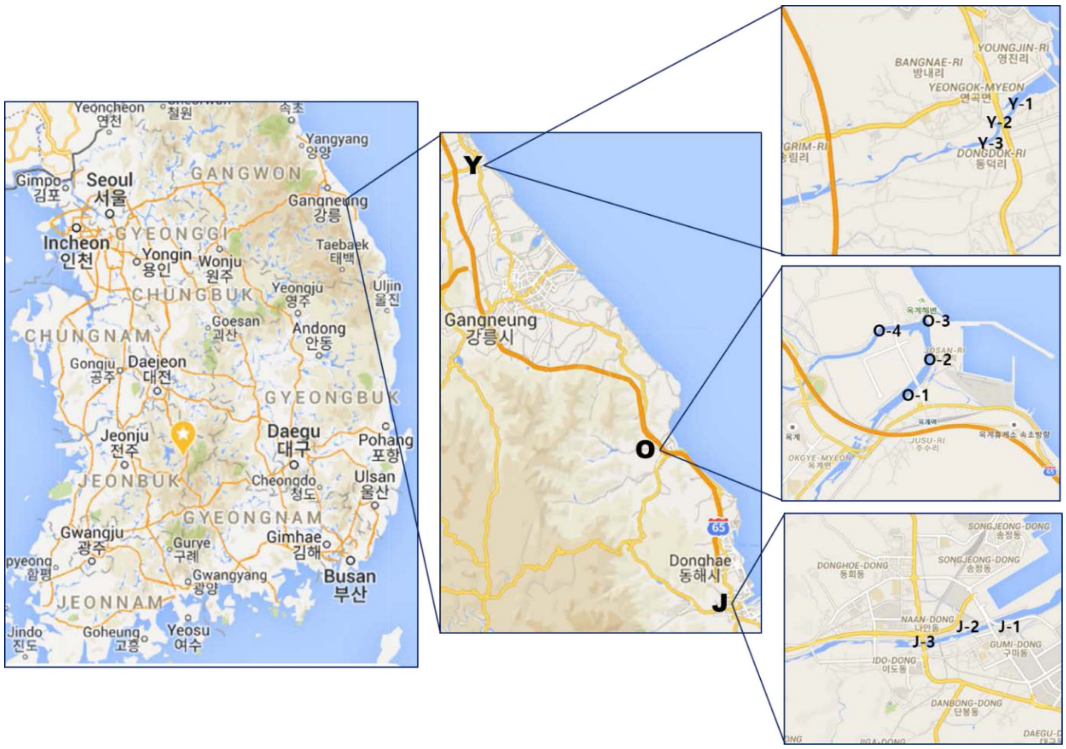

Fig. 1. Location of Yeongok stream (Y), Okgye-myeon (O), and Jeon stream (J) for the collection of water and fish samples.

magnesium smelting plant in June 2013. Sites Y1-3 and J1-3 sites were in Yeongok Stream (Gangneung) and Jeon Stream (Donghae), respectively.

The water and fish samples were collected in spring (April 7 and 8) and summer (July 3-5) of 2017. Three water samples were collected at each site from 50 cm below the water surface in 250-mL amber glass bottles without headspace, to which about 25 mg of sodium thiosulfate was immediately added. All water samples were stored in an ice box, transported to the lab, and analyzed within 48 h. In addition, the fish species dace (*Tribolodon hakonensis*) and gray mullet (*Mugil cephalus*) were captured using a fishing net near the water sampling sites. These two species were the dominant at the sampling sites, and dace was the most common fish at all sites. The length of the fish collected ranged from 25.0 to 46.0 cm, and the weight ranged from 85 to 872 g. Each fish was dissected on site using a filleting knife, scalpel, and forceps, after which muscle, liver, gill, and kidney tissue samples were obtained and placed in a zipper bag or a 15-mL falcon tube. The anatomical samples were stored in a dry ice box, transported to the lab, and kept in a freezer (-20 °C) until analysis.

2.3. Sample preparation

2.3.1. Water

Water samples were filtered through a 0.2-μm nylon membrane filter (Whatman, US) and all samples (50 mL) were spiked with 1 μL of SS solution. The pH was adjusted to approximately 2 using 6 N HCl solution before extraction. MTBE (50 mL) was added to the sample and the analytes were extracted under vigorous shaking for 15 min. The supernatant was transferred to another container and the extraction was repeated twice. The combined extract (100 mL) was dried over 3 g of sodium sulfate and concentrated to dryness using a rotary vacuum evaporator (Hahnshin S&T, Gimpo, Korea) and nitrogen gas. The concentrate was reconstituted with 1 mL of ultrapure water and then the derivatization process was followed.

2.3.2. Fish

For sample pretreatment, a method for extracting phenols from a fish sample after acid digestion was used.[19] One gram was aliquoted from each tissue (muscle, gills, kidney, and liver) and homogenized using SHG-15A homogenizer (SciLab, Seoul, Korea). The sample was placed in a 40-mL glass bottle and spiked with 200 μL of 1 mg/L SS solution. The sample was digested by adding 100 μL of 12 N H_2SO_4 and sonicated with 15 mL of ethyl acetate for 30 min. The supernatant was transferred to a 15-mL falcon tube and centrifuged at 11,099 × g for 10 min. Extraction using ethyl acetate was repeated twice. The combined extract (30 mL) was dried over 1 g of sodium sulfate and concentrated to dryness using a vacuum rotary evaporator and nitrogen gas. The concentrate was reconstituted with 1 mL of ultrapure water and then the derivatization process was followed.

2.3.3. Derivatization to phenyl acetates

The phenols were derivatized via acetylation prior to GC-MS analysis. First, 1 mL of the concentrate was added to 0.5 mL of 5 % aqueous potassium carbonate solution and 0.1 mL of acetic anhydride. The mixture was vigorously shaken for 20 s using a vortex mixer and left at room temperature for 10 min. The analytes were extracted with 1 mL of *n*-butyl acetate and 10 mg of sodium chloride via vortex mixing for 1 min. The supernatant was filtered through a Pasteur pipette filled with a small amount of sodium sulfate on cotton wool and placed in a 2-mL vial. Following this, 1 μL of the sample was injected into the GC-MS.

2.3.4. Instrumental analysis

The prepared samples were analyzed using a gas chromatograph (7890A) coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) under the GC-MS conditions presented in *Table* 1. Peak separation was conducted using a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies) with He as the carrier gas at 1 mL/min. The following oven temperature program was employed: initiated at 40 °C and held for 1 min; increased to 110 °C at 10 °C/min; increased to 140 °C at 2 °C/min; increased to 260 °C at 20 °C/min;

Table 1. GC-MS parameters used for the quantitative determination of phenols in the water and fish tissue samples

GC (7890A GC, Agilent Technology, Inc.)	Carrier gas, flow rate			He, 1 mL/min
	Column			Agilent DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm)
	Oven temperature program			40 °C, hold for 1 min → to 110 °C at 10 °C/min → to 140 °C at 2 °C/min → to 260 °C at 20 °C/min → to 280 °C at 30 °C/min, hold for 10 min
	Inlet port temperature.			280 °C
	Injection mode			Splitless
	Injection volume			1 μL
MS (5975C Inert MSD, Agilent Technology, Inc.)	Transfer line temperature			280 °C
	Ionization mode			Electron impact (EI), 70 eV
	Ion source temperature			230 °C
	Quadrupole temperature			150 °C
	Selected ions (m/z) for phenyl acetates	Analyte	Retention time (min)	Fragment ions
		P	13.704	94, 136, 66
		C (SS)	15.311	107, 108, 150
		24DMP	17.263	107, 122, 164
		4C3MP	18.899	107, 142, 184
		24DCP	19.071	162, 164, 204
		2NP	19.654	139, 181
		246TCP	20.421	196, 198, 240
		4NP	20.799	139, 181
		PCP	24.195	266, 264, 308

increased to 280 °C at 30 °C/min; and then held for 10 min. Samples (1 μL) were injected into the injection port, which was maintained at 280 °C in splitless mode using a 7693A autosampler (Agilent Technologies). The temperatures of the MS transfer line, the ion source, and the quadrupole were 280 °C, 230 °C, and 150 °C, respectively. The mass spectrometer was operated in the selected ion monitoring (SIM) mode for quantitative analysis. The fragment ions of the phenyl acetates are presented in *Table* 1. In the chromatogram, the peaks of the target occurred at 13.704 min for P, 17.263 min for 24DMP, 18.899 min for 4C3MP, 19.071 min for 24DCP, 19.654 min for 2NP, 20.421 min for 246TCP, 20.799 min for 4NP, and 24.195 min for PCP, while that for the SS was at 15.311 min.

2.3.5. Method validation

The proposed method for the quantitative determination of phenols was validated based on measures of linearity, detection limits, accuracy, and precision. Calibration curves were drawn at five points within the 0.3–4.0 μg/L range for the water samples and the 10–600 μg/kg range for the fish samples. The coefficient of determination (r^2) for the calibration curves was used to assess their linearity (>0.99). The method detection limit (MDL) and limit of quantification (LOQ) were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. Accuracy and precision were evaluated using the recovery and repeatability (relative standard deviation, RSD), respectively.

2.4. Bioconcentration factor

Bioconcentration factors (BCF, L/kg) were calculated by dividing C_{fish} by C_{water}, where C_{fish} is the concentration in the fish (μg/kg) and C_{water} is the mean concentration in water at each sampling site (μg/L). The values were expressed as log BCF.

2.5. Risk assessment

An ecological risk assessment for chemical exposure

within an aquatic environment was conducted following the risk quotient (RQ) method.[20] The RQ was expressed as the ratio of the measured environmental concentration (MEC) and predicted no-effect concentration (PNEC). An RQ less than 1 suggests that there is no significant risk associated with the environmental presence of the chemical of interest, whereas a value greater than 1 suggests that further environmental risk characterization or management of the chemical is necessary.

Of the phenols examined, only P and 4NP were included in the risk assessment because their PNEC was available in a database or from past research. The PNEC for P in freshwater is given as 0.008 mg/L by the European Chemicals Agency,[21] which was obtained using the assessment factor (AF = 10) method for extrapolation. The PNEC used for 4NP was 58 μg/L, which is an interim guideline calculated based on the median lethal concentration and the chronic no-observed-effect concentration (NOEC) for nine freshwater fish species.[22]

2.6. Statistical analysis

The samples were collected from the sites (Y1-3, O1-4, and J1-3) in both spring and summer. To confirm regional and seasonal differences, IBM SPSS Statistics version 24 (Armonk, NY) was used for the statistical analysis of the data. Student's *t*-tests were used to compare the mean concentrations for the water samples between spring and summer at a significance level of 1 %. An analysis of variance (ANOVA) was conducted for the differences in the mean concentration of compounds in the water samples between the sites at a significance level of 5 %.

3. Results and Discussion

3.1. Method optimization

The extraction conditions for the proposed method, including the extraction solvent, solvent volume, and time, were also optimized to extract all target compounds with a high recovery rate. In this process, three organic solvents were tested: DCM, ethyl acetate, and MTBE. The eight target phenols were spiked in duplicate into purified water at 4 μg/L and into fish muscle at 100 μg/kg. MTBE exhibited the strongest chromatographic response for the water samples, whereas ethyl acetate produced the strongest response for the fish samples (*Fig.* 2). In contrast, the use of DCM led to unclear phase separation and was unsuitable for both sample types. MTBE and ethyl acetate were thus selected as the extraction solvents for the water and fish samples, respectively, because of their higher sensitivity and precision.

The solvent volume and extraction time were also compared for both sample types. Water and fish samples were extracted with 50 mL for 15 minutes and 15 mL for 30 minutes, respectively, and single extraction and repeated extraction methods were

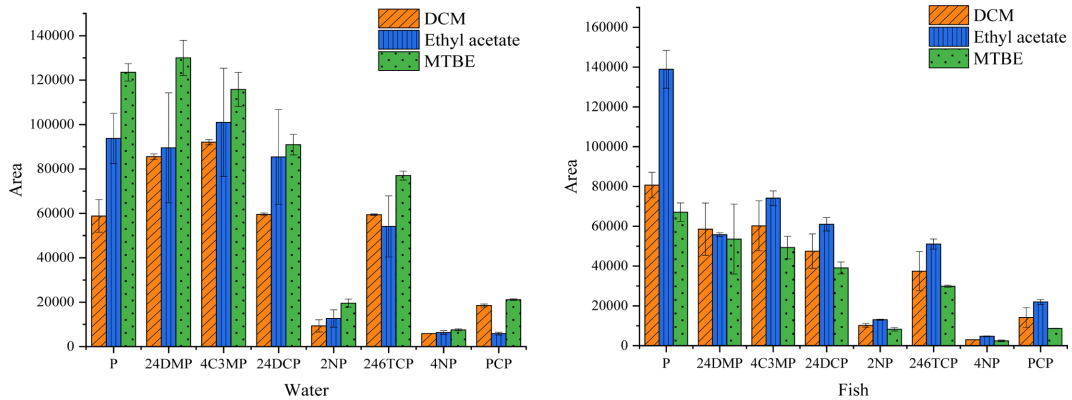

Fig. 2. Comparison of the chromatographic area for the extraction of phenols in water and fish samples. For the water samples, the extraction process was repeated twice for 15 minutes using 50 mL of each solvent. For the fish tissue samples, phenol extraction was repeated twice for 30 minutes using 15 mL of each solvent after acid decomposition.

Table 2. Method validation for the detection of phenols in the water and fish samples

Compound name	Water					Fish				
	r^2	MDL (μg/L)	LOQ (μg/L)	Recovery (RSD) (%, n = 3)		r^2	MDL (μg/kg)	LOQ (μg/kg)	Recovery (RSD) (%, n = 3)	
				0.3 μg/L	4 μg/L				100 μg/kg	200 μg/kg
P	0.9967	0.120	0.38	102 (5.2)	88.7 (1.5)	0.9960	9.4	30	108 (5.7)	84.3 (3.8)
24DMP	0.9992	0.044	0.14	83.2 (6.2)	85.5 (4.0)	0.9991	4.4	13	76.5 (6.7)	85.4 (2.9)
4C3MP	0.9995	0.043	0.14	95.3 (4.8)	98.7 (5.8)	0.9962	6.8	22	95.0 (5.1)	103 (7.0)
24DCP	0.9996	0.030	0.095	82.5 (4.3)	85.7 (3.6)	0.9970	8.1	26	91.6 (5.5)	98.8 (7.2)
2NP	0.9973	0.068	0.21	87.5 (4.6)	83.8 (5.9)	0.9969	12	39	110 (5.3)	97.9 (11)
246TCP	0.9996	0.050	0.16	92.0 (7.5)	91.1 (3.8)	0.9971	9.4	30	93.9 (5.0)	107 (8.4)
4NP	0.9978	0.042	0.13	115 (9.1)	111 (4.0)	0.9977	12	39	80.7 (5.7)	102 (6.3)
PCP	0.9960	0.076	0.24	114 (8.2)	104 (3.1)	0.9965	6.7	22	100 (6.0)	96.2 (9.4)

Table 3. Concentrations (μg/L) of phenols in the water samples

Season	Site	P	4C3MP	246TCP	4NP	Total
Spring	Y (n = 3)	3.07 ± 0.07	0.279 ± 0.172	0.238 ± 0.124	1.00 ±0.99	1.15 ± 1.28
	O (n = 4)	3.02 ± 0.06	0.139 ± 0.101	0.075 ± 0.100	0.548 ± 0.375	0.946 ± 1.266
	J (n = 3)	3.23 ± 0.11	0.275 ± 0.258	0.255 ± 0.237	1.54 ± 1.48	1.33 ± 1.43
	Total	3.10 ± 0.11 (2.97–3.32)	0.222 ± 0.173 (<MDL[a)]–0.537)	0.178 ± 0.165 (<MDL–0.502)	0.982 ± 0.969 (<MDL–3.13)	1.12 ± 1.29
Summer	Y (n = 3)	1.42 ± 0.24	0.020 ± 0.000	0.058 ± 0.040	0.020 ± 0.000	0.379 ± 0.635
	O (n = 4)	0.908 ± 0.576	0.500 ± 0.466	0.468 ± 0.466	1.55 ± 2.24	0.856 ± 1.167
	J (n = 3)	1.78 ± 0.41	0.090 ± 0.000	0.058 ± 0.040	0.271 ± 0.054	0.549 ± 0.765
	Total	1.32 ± 0.56 (<MDL–2.11)	0.233 ± 0.355 (<MDL–1.07)	0.222 ± 0.343 (<MDL–0.984)	0.706 ± 1.488 (<MDL–4.91)	0.621 ± 0.919
Grand total		2.21 ± 0.99 (<MDL–3.32)	0.227 ± 0.272 (<MDL–1.07)	0.200 ± 0.263 (<MDL–0.984)	0.844 ± 1.230 (<MDL–4.91)	0.871 ± 1.144

[a)] <MDL: Below the method detection limit.

compared. As a result, a method of repeated extraction twice was selected to sufficiently recover phenols from two types of samples.

3.2. Method validation

The proposed method was validated in terms of its linearity, MDL, LOQ, accuracy, and precision. *Table* 2 presents the results for the water and fish samples, respectively. The coefficient of determination (r^2) for the linearity of the calibration curves was above 0.996 for both methods and all compounds. The MDLs ranged from 0.03 μg/L to 0.12 μg/L for the water samples and 4.4 μg/kg to 12 μg/kg for the fish samples, while the LOQs ranged from 0.095 μg/L to 0.38 μg/L for the water samples and 13 μg/kg to 39 μg/kg for the fish samples. Accuracy and precision were measured as the recovery (%) and the RSD (%), respectively. These were found to be 82.5–115 % and 1.5–9.1 %, respectively, at concentrations of 0.3 μg/L and 4 μg/L for the water samples and 76.5–110.0 % and 2.9–11.0 %, respectively, at 100 μg/kg and 200 μg/kg for the fish samples. These results thus confirmed that the method proposed in this study is suitable for quantitative analysis.

3.3. Concentrations of phenols in water

The concentration of phenols was measured for 20 water samples collected in spring and summer (*Table* 3). In Korea, more than 60% of annual precipitation is concentrated in summer (July and August).[23] Therefore,

by comparing the two seasons, the effect of rainfall on the concentrations of phenols in water could be observed. P was detected in 19 of the samples, 4C3MP in 10, 24DCP in 2, 246TCP in 8, and 4NP in 14. PCP was not detected in any sample. Therefore, only P, 4C3MP, 246TCP, and 4NP were included in the data analysis. In the spring samples, the concentrations of P, 4C3MP, 246TCP, and 4NP were 3.10 ± 0.11 μg/L, 0.222 ± 0.173 μg/L, 0.178 ± 0.165 μg/L, and 0.982 ± 0.969 μg/L, respectively, compared to 1.32 ± 0.56 μg/L, 0.233 ± 0.355 μg/L, 0.222 ± 0.343 μg/L, and 0.706 ± 1.488 μg/L, respectively, in the summer samples.

Seasonal and regional differences in the concentrations of the four compounds were tested using Student's *t*-tests and one-way ANOVA, respectively. A significant seasonal difference was found only for P (p = 0.000), whereas there was no difference for the other phenols. Similarly, no regional differences were found for any of the phenols. The seasonal pattern observed for P in the present study was in accordance with a previous study in Brazil, which found that the concentrations of phenols in surface water were higher in winter than in summer, which was associated with differences in precipitation levels (278 mm in summer and 13 mm in winter[24]). Indeed, the concentrations of organic pollutants can be diluted by rainfall or biodegraded in warm conditions.[25]

In the present study, phenols were detected in the range of < MDL–4.91 μg/L, with P and 4NP exhibiting the highest detection rates and concentrations. These concentrations are within the ranges reported for phenols in previous research. For example, in natural water, P has been reported to occur at concentrations of 0.01–5.60 μg/L in Poland and the Netherlands, and nitrophenols have been reported to be present at concentrations of 0.04–10 μg/L in Spain and Japan.[6] Statistically, the concentrations were similar in the accident and control areas, and based on the previously reported background concentrations of phenols in natural waters, it is possible that the P released in the spill at the magnesium refining plant in Gangneung in 2013 no longer affected the phenol levels of the water bodies within the area.

The P in natural surface water can originate from the decomposition of natural organic matter, such as humic substances from plants.[1] It also can be introduced anthropogenically via industrial waste disposal processes or chemical spills. For example, in 1991, more than 30 tons of P were spilled into the Nakdong River in Korea, with P entering the water purification plant and turning into chlorophenol, leading to odor problems.[26] Substituted phenols may also form in the reaction between P and substituent anions. For instance, nitrate ions in water can react with P under sunlight to produce 2NP and 4NP over a wide pH range.[27]

In the present study, the levels of P and substituted phenols in natural domestic water bodies were investigated. Although the concentration of chlorophenols detected in the water samples was low in the present study, the P in aquatic systems can be present in raw water used for drinking or react with the chloride in seawater to produce chlorophenols. Given that the lowest-observed-adverse-effect-level (LOAEL) of 2CP is 50 mg/kg-day and that of 24DCP is 3.0 mg/kg-day, compared to 360 mg/kg-day for P,[28-30] chlorophenols are considered more toxic than P. In particular, PCP is classified as a Group B2 carcinogen,[31] thus it needs to be carefully controlled even if its detection rate is low.

3.4. Concentrations of phenols in fish tissue

Aquatic organisms can accumulate phenols in their body tissues via oral consumption, inhalation, and dermal absorption.[32] *Table* 4 summarizes the concentrations of phenols found in the fish tissue samples in the present study. P, 24DMP, 2NP, and 4NP were detected above the LOQ for all of the tissue types, with the exception of 24DMP in kidney tissue. The target phenols were found to range from < MDL to 407 μg/kg, from < MDL to 26.0 μg/kg, from < MDL to 161 μg/kg, and from < MDL to 870 μg/kg for P, 24 DMP, 2NP, and 4NP, respectively. P and 4NP, which were dominant in the water samples, also exhibited the highest concentrations in the tissue samples. In particular, 4NP was observed at its highest levels in kidney tissue (350 ± 157 μg/kg) in spring, whereas P was the most abundant in muscle (125 ± 22 μg/kg) and gill tissue (130 ± 122 μg/kg) in spring.

Table 4. Concentration (μg/kg) of phenols in the fish tissue samples (muscle, gill, liver, and kidneys)

Target	Site	Spring					Summer				
		Sample size	Muscle	Gill	Liver	Kidney	Sample size	Muscle	Gill	Liver	Kidney
P	Y	1	143	37.2	62.5	<MDL[a)]	-[b)]	-	-	-	-
	O	3	101 ± 77	84.6 ± 41.9	127	19.7	1	142	38.2	19.7	30.6
	J	2	132 ± 65	269 ± 195	88.7 ± 30.7	27.5 ± 11.0	1	92.8	94.5	19.7	19.7
	Total	6	125 ± 22 (52.4–189)	130 ± 122 (36.3–407)	92.6 ± 32.3 (62.5–127)	23.6 ± 5.5 (<MDL–35.3)	2	117 ± 35 (92.8–142)	66.3 ± 39.8 (38.2–94.5)	19.7 ± 0.0 (19.7–19.7)	25.2 ± 7.7 (19.7–30.6)
24DMP	Y	1	26.0	<MDL	<MDL	<MDL	-	-	-	-	-
	O	3	8.70	11.4 ± 4.71	8.70	<MDL	1	8.70	<MDL	<MDL	<MDL
	J	2	8.70	8.70	8.70	<MDL	1	<MDL	<MDL	<MDL	<MDL
	Total	6	14.4 ± 10.0 (8.70–26.0)	10.1 ± 1.9 (<MDL–16.9)	8.70 (<MDL–8.70)	<MDL	2	8.7 (<MDL–8.70)	<MDL	<MDL	<MDL
2NP	Y	1	68.9	<MDL	58.4	<MDL	-	-	-	-	-
	O	3	25.5	<MDL	25.5	111 ± 71	1	25.5	25.5	40.3	25.5
	J	2	25.5	71.4	25.5	25.5	1	25.5	<MDL	<MDL	<MDL
	Total	6	40.0 ± 25.1 (25.5–68.9)	71.4 (<MDL–71.4)	36.5 ± 19.0 (25.5–58.4)	68.3 ± 60.5 (<MDL–161)	2	25.5 ± 0.0 (25.5–25.5)	25.5 (<MDL–25.5)	40.3 (<MDL–40.3)	25.5 (<MDL–25.5)
4NP	Y	1	73.2	141	87.7	<MDL	-	-	-	-	-
	O	3	25.5	131 ± 25.6	92.4	239	1	25.5	80.5	<MDL	870
	J	2	25.5	81.3 ± 39.2	40.3 ± 21.0	461	1	25.5	180	<MDL	<MDL
	Total	6	41.4 ± 27.5 (25.5–73.2)	118 ± 32.0 (53.6–160)	73.5 ± 28.8 (25.5–92.4)	350 ± 157 (<MDL–461)	2	25.5 ± 0.0 (25.5–25.5)	130 ± 70 (80.5–180)	<MDL	870 (<MDL–870)

[a)] <MDL: Below the method detection limit.
[b)] Samples at site Y were not taken in summer

Although 24DMP and 2NP were not detected in water, fish samples were detected as follows. 24DMP was found to range from < MDL to 26.0 μg/kg in fish muscle, from < MDL to 16.9 μg/kg in gill, and from < MDL to 8.70 μg/kg in liver. 2NP was found to range from 25.5 to 68.9 μg/kg in fish muscle, from 25.5 to 71.4 μg/kg in gill, from < MDL to 58.4 μg/kg in liver, and from < MDL to 161 μg/kg in kidney.

Previous research has shown that the bioaccumulation of phenols in the body tissue of fish appears to be dependent on the type of phenol and the fish species. For example, accumulated P was found to be highest in the liver of Nile tilapia (*Oreochromis niloticus*), followed by the gills and muscles, after 16 weeks of exposure, with its concentration in tissue increasing with its concentration in water.[12] On the other hand, yellowtail horse mackerel (*Trachurus novaezelandiae*) rapidly accumulated phenols in muscle tissue but not in the liver.[33] In 2015, the concentration of P in water was found to be 2.51–7.51 μg/L in the same areas as the sites monitored in this study, while its concentration in the renal tubules of dace and gray mullet was 216 ± 44 μg/kg and 387 ± 147 μg/kg, respectively.[19,34] In this study, the log BCF values for P and 4NP were calculated. The values for P were 1.3–2.2 for muscle, 1.1–2.1 for gills, 1.0-1.6 for liver, and 0.8–1.5 for kidneys. The values for 4NP were 1.2–2.0 for muscle, 1.5–2.8 for gills, 1.2–2.2 for liver, and 2.5–2.7 for kidneys. P was the most concentrated in muscle, followed by gills. It was also confirmed that 4NP was the most concentrated in gills and kidneys. However, this study has limitations because of a small number and species of fish inhabiting each sampling site. Therefore, it was not easy to compare bioaccumulation factors by regions, species, and tissues. In the future, a more extended study is needed to compare the bioaccumulation factors of phenols.

Mono-nitrophenols are easily metabolized to glucuronide and sulfate conjugates and mostly excreted in the urine within 24–48 h.[7] However, in this study, P and 4NP were identified as the major phenols in water and fish tissue. Phenols were found to be present at μg/L levels in both spring and summer, thus the

aquatic life in the area is considered to be chronically exposed. It has been reported that phenols can accumulate to the mg/kg level in fish tissue, including the bladder or kidney, while the concentration of P in fish kidneys was significantly higher after exposure for 10-30 days than for the control group.[35,36] Therefore, it is considered that aquatic organisms are chronically exposed to phenols in Korea, and the need for management and regulation must be confirmed by assessing the risks.

3.5. Risk assessment

Phenols in the aquatic environment have the potential to affect not only aquatic organisms but also humans. In the present study, the ecological RQ for fish was calculated using the concentrations of phenols in water measured in this study. *Table* 5 presents the MEC for P and 4NP in the water samples by site. The average MEC for P and 4NP was found to be 2.21 μg/L and 0.844 μg/L, respectively, while the 95th percentile MEC, considered the worst-case scenario, was 3.25 μg/L and 3.21 μg/L, respectively. The PNEC for P and 4NP was reported to be 8 μg/L and 58 μg/L, respectively. *Table* 5 also displays the ecological RQ for the chronic exposure of fish to P and 4NP, with an RQ exceeding 1.0 indicating a potential stressor. Including all sites, the average RQ was 0.276 and 0.015 for P and 4NP, respectively, whereas the 95th percentile RQ was 0.406 and 0.055, respectively.

Ecological risk assessment is conducted to identify stressors that can have an adverse ecological effect and to assess the risk posed to ecological assessment endpoints.[37] In previous studies related to the ecological risk assessment of phenols, Zhong *et al*. (2010) found that 2NP was dominant in Zhushanhu, with surface water levels of up to 1.55 μg/L, with an RQ exceeding 0.3.[38] In accordance with the Water Environment Research Foundation of Alexandria,[39] a potential stressor is identified as a concern when its RQ is higher than 0.3 in consideration of potential chemical interactions and accumulation. In addition, Ramos *et al.* (2021) classified RQs into high risk (RQ > 1), medium risk (1 ≥ RQ ≥ 0.1), and low risk (0.01 > RQ), with 4NP detected at a concentration of 4.47–25.40 μg/L in raw water, representing high and medium risk for acute and chronic exposure, respectively.[24] This classification method has also been used for ecological risk assessment of phenols in Weihe River in China, with 24DCP and PCP detected at high risk levels and other phenols such as P, 4NP, and 2CP found at medium risk levels.[40] In this study, the RQ for P and 4MP was less than 1, indicating that they did not pose a high risk. However, they are considered to be present at non-negligible levels because they can potentially be harmful to fish. In particular, the aquatic environment may be adversely affected if the levels of organic matter or nitrogen compounds from natural or anthropogenic sources increase and the concentration of phenols within the aquatic environment increase. Therefore, it is necessary to control and manage the background concentration of phenols in the domestic environment.

4. Conclusions

In the present study, a method for the analysis of phenols in water and fish tissue samples using GC-MS was developed and tested at three sites in Gangwon State. The method was sufficiently sensitive to detect

Table 5. Measured exposure concentrations (MEC) and ecological risk quotients (RQ) for phenol (P) and 4-nitrophenol (4NP) at each site

Compound	Statistics	MEC (μg/L)				RQ_{fish}			
		Site Y	Site O	Site J	All sites	Site Y	Site O	Site J	All sites
P	Average	1.97	2.50	2.25	2.21	0.246	0.313	0.281	0.276
	95th percentile	3.08	3.30	3.13	3.25	0.385	0.413	0.391	0.406
4NP	Average	1.05	0.907	0.510	0.844	0.018	0.016	0.009	0.015
	95th percentile	3.53	2.67	1.75	3.21	0.061	0.046	0.030	0.055

eight phenols, with P and 4NP the two major compounds found in both the water and fish samples, with maximum concentrations of 4.91 μg/L and 870 μg/kg, respectively. Regional differences in the concentration of the phenols were not observed, suggesting that a P spill near Site O do not differ from other sites in P or its derivatives. However, the background phenol levels suggest that these compounds occur naturally, thus their formation mechanisms need to be elucidated in the future. P and 4NP were found in the fish tissue samples, indicating they are bioaccumulated in the body following long-term exposure in water. Larger sample sizes are needed to determine the bioconcentration of phenols in aquatic ecosystems. Even though the phenol levels were calculated to be below the estimated PNEC for fish, it is necessary to continue to study these compounds because they are naturally present and can be introduced anthropogenically. Additionally, extensive research on the distribution and formation of these pollutants in surface and drinking water needs to be conducted.

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